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Organ injury study and the mortality rate assessment in a cecal slurry dose-dependent peritonitis in a rabbit model

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ABSTRACT

Introduction: Sepsis is a life-threatening medical crisis in which the body responds to infections extremely. The present study is aimed to assess the extent of mortality in cycle slurry, dose-dependent peritonitis in the rabbit model. *Method and Materials*: The present study was performed on male polish rabbits. At least 10 days before the experiment, the rabbits were placed in a controlled environment and received standard amounts of water, food, and libitum. Anesthesia of 6 donor rabbits was performed by intramuscular injection of 5 mg/kg Xylasein and 35 mg/kg ketamine. *Result:* The total number of lymphocytes, neutrophils, and white blood cells (WBC) in the groups of 15 and 20 ml/kg was less than the groups of 5 and 10 ml/kg, no significant differences were observed between the groups (P> 0.05). The number of platelets was also higher in the 20 mL/kg group in regard to the sham group. All of the sepsis groups had lower concentrations of plasma albumin than the sham group. Increased doses of cecal slurry were associated with an increase in the ALT and BUN concentrations. Higher concentrations of ALT and BUN were observed in the sepsis groups than in the sham group. Although the samples in the 15 and 20.0 mL/kg groups showed higher creatinine concentrations than those in the sham group, the difference was insignificant. *Conclusion:* Based on the DCFH-DA concentrations and blood chemistry analysis, the cecal slurry model used in this study produced dose-dependent, multiple organ injuries and was associated with ROSs generation.

Keyword: Rabbit, Cecal Slurry, Peritonitis, Organ Injury

1. INTRODUCTION

Sepsis refers to a condition where the severe response of the body to a life-threatening infection damages its tissue and can cause the failure of organs and death. Therefore, sepsis is a problem of the existence of infection in the body. The body fights the infection by releasing chemicals into the bloodstream, triggering inflammatory reactions across the body (Hildebrand et al., 2013) which can cause organ changes, damage, and paralysis. The progression of sepsis to septic shock can cause a severe drop in blood

pressure, which is fatal. Although sepsis can occur in everyone, older adults and those with weakened immune systems are more prone to this complication. If the sepsis is treated in the early stages, usually by consuming antibiotics and intravenous fluids, the chance of survival considerably increases. Understanding the pathophysiology of organ failure in sepsis is of great importance not only for better management and treatment of the disease but also for developing new treatments (Hamilton et al., 2012). Clinical evaluation and monitoring are mainly applicable for the six major organ systems including respiratory, hematological, neurological, renal, hepatic, and cardiovascular systems, while it is less available for other systems such as the intestine (Lee et al., 2014). There is increasing evidence regarding the role of microcirculation in sepsis and research tools have been developed with the ability to visualize the microcirculation at the bedside. The development of appropriate animal models can help to develop new agents for adjuvant therapy of sepsis in human conditions. However, many animal models developed for this purpose have led to misleading results.

Several factors can be responsible for these discrepancies between the results of human clinical trials and those obtained from animal models (Lee et al., 2014; Ii et al., 1997). An important factor may be different alterations in gene expression as a result of infection or trauma between humans and mice, an animal model commonly used for experiments. Besides, unlike humans, who are highly sensitive to the toxic effects of bacterial lipopolysaccharide, many animals, such as baboons and mice, are significantly resistant to them. Recent studies have used animal models to investigate sepsis and identify new therapeutic agents for sepsis. However, generalizing such preclinical results to clinical use requires careful consideration and should be viewed with some skepticism. Several animal models have been developed for sepsis, including cecal ligation and incision model, the cecal ligation and puncture (CLP) model, and the lipopolysaccharide (LPS) injection model, all of which suffer from some degrees of limitations and biases in comparison with human sepsis (Gentile et al., 2014, Cross et al., 1993).

One of the methods to create sepsis in animals is the model of cecal slurry peritonitis, in which the cecal contents from other animals are injected intraperitoneally to induce polymicrobial sepsis in the body of the studied animals (Dejager et al., 2011; Dyson and Singer, 2009; Fink, 2002). It is demonstrated that the cecal slurry model causes physiological and hemodynamic alterations that are associated with sepsis in human bodies. This model was originally developed as an alternative polymicrobial model of sepsis. The present study is aimed to examine different organ injuries and dose-dependent mortality in the cecal slurry peritonitis model using the rabbit model of sepsis (Marshall et al., 2005; Saito et al., 2003; Wood et al., 1993).

2. MATERIALS AND METHODS

This study was performed based on current knowledge and accepted scientific principles and a complete review of existing scientific sources and prior laboratory investigations. In this study, the ethical principles of working with laboratory animals were fully observed. The methods of this study are in accordance of the National Institutes of Health Guidelines and also are approved by the Institutional Animal Care and Use Committee of our institution (Lee et al., 2011). The present study was performed on male polish rabbits with a weight between 1.78 and 2.00 kg from July to September 2020. At least 10 days before the experiment, the rabbits were placed in a controlled environment and received standard amounts of water and food, libitum. A modified model of cecal slurry peritonitis was used to induce polymicrobial sepsis. 5 mg/kg Xylasein 2% and 35 mg/kg ketamine 10% (Alfasan Inc., Woerden, Netherland) were injected intramuscularly to induce anesthesia of 6 donor rabbits. Cecum was extruded after performing midline laparotomy. The Anti-mesenteric surface of the cecum was incised by 1.2 cm and pressure was applied on the cecum to defecate (Ambroze et al., 1991;, Rittirsch et al., 2009; Shrum et al., 2014). After being collected and weighed, the feces were immediately diluted with a 7% dextrose solution in a ratio of 1:4. To obtain a homogeneous suspension, the cecal slurry of the donor rabbits was blended and vortexed before administration. The prepared cecal slurry for each experiment was used after 4 h of preparation. Halothane aspiration (a mixture of 1% halothane in 40% oxygen) was used to anesthetize the rabbits.

The rabbits' abdomens were shaved and treated with ethanol 80%. Then a 1.2 cm midline incision was made to administer the required cecal slurry into the peritoneal cavity based on the dose group. Then, 35 mL/kg normal saline solution 1.1% was administered into the peritoneal cavity. Afterward, 10 mg/kg of enrofloxacin was injected intramuscularly, and another subcutaneous injection of 30 mL/kg saline solution 1.1% was performed for fluid resuscitation. Identical procedures were performed for sham-treated samples except that they received no cecum slurry. The cecal slurry samples were smeared on a salmonella-Shigella agar plate, a MacConkey agar plate, and a blood agar plate. The agar plates were then incubated for 24 hours at a temperature of 37°C. The cecal slurry was analyzed for its composition and colony-forming units (CFU) per ml. As described previously, sepsis was induced in 8 rabbits using 7.0 mL/kg of cecal slurry. Abdominal aortic blood samples were collected from rabbits after 24 hours of sepsis induction, and 4 mL blood samples were prepared from each rabbit and introduced into the BD

BACTEC™ Plus Aerobic medium and BD BACTEC™ FX blood culture (Becton Dickinson & Company, Shannon, Ireland) and incubated for 96 hours at a temperature of 37°C. Microbiological analysis of the samples was conducted after the incubation.

According to the volume of the cecum, slurry received, the rabbits were separated into four categories, including groups of 5, 10, 15, and 20 mL/kg. To study the survival of the rabbits, they were followed for 21 days from the sepsis induction (n = 20 for the group of 15 mL/kg, n = 30 for the group of 10 mL/kg, and n = 10 for the groups of 5.0 and 20 mL/kg). Rabbits who survived after the 21-day follow-up period were sacrificed by exsanguination and underwent autopsies. Rabbits were euthanized by exsanguination after 8 hours of sepsis induction (n = 5 in the sham group and n = 10 in all four treatment groups). After laparotomy, the blood sample of each rabbit's abdominal aorta was removed using a heparin syringe (Hi Trap® Heparin High-Performance GE17-0407-01 Cytiva). Immediately after preparing the samples, a complete blood count and arterial blood gas analysis were accomplished. Another part of the blood samples was centrifuged at a speed of 3000 rpm for 20 minutes at a temperature of 5°C to separate plasma. The obtained plasma samples were stored at −73°C to be used for blood chemistry profile analyses. Lungs, livers, and kidneys of the rabbits were also harvested and stored at −70°C for further analyses. HemataStat II® Microhematocrit Centrifuge HemoScreen™ (Labcompare, South SF, USA) was employed to perform complete blood count and Class 2 Device Recall Stat Profile Critical Care Xpress (CCX and CCX+, Diamond Diagnostics Inc., USA) was used for arterial blood gas (ABG) analyses.

The analysis of plasma chemistry profile including albumin, creatinine, blood urea nitrogen (BUN), and alanine aminotransferase (ALT) concentrations were performed using a Vet Scan VS2 (Randox Laboratories Ltd.) to evaluate the severity of organ injuries. As a successor marker to produce reactive oxygen species and the levels of dichloro-dihydro-fluorescein diacetate (DCFH-DA) were determined in kidney, liver, and lung tissues (Thermo fisher scientific, Waltham, MA, US). Values are represented as MFI per mg of tissue and log-rank test and Kaplan–Meier curves were used for survival rate assessments.

Comparison of variables was done by performing an analysis of variance with post hoc analysis, and continuous variables were displayed as mean ± standard deviation. SPSS V25 software (IBM Corp., New York, NY) was applied to perform statistical analyses. P values lower than 0.05 were regarded statistically significant. This project has been approved by the Research Council of the Medical School with the ethical code number of IR.RHC.REC1398.084.

3. RESULTS

Table 1 shows the relative frequency of the microorganisms isolated from the cecal slurry. Firmicutes were the predominant phylum of bacteria within the completely gastrointestinal tract. In second place were Proteobacteria in the foregut and Bacteroidetes in the hindgut. A comparison of the live and total bacteria profiles showed a shift in the ranking of predominant phyla in the foregut. However, these changes were less obvious in the hindgut and slight variations were observed in the relative frequency of Bacteroidetes and Firmicutes. This finding is an indication of the more substantial interference of dead bacteria with the microbiota in the foregut. All 8 blood cultures obtained from the rabbits contained bacteremia. The isolation of *E. coli, S. faecium, and C. Albicans* were performed for each animal (Table 2). Following 24 hours of the induction of sepsis, blood cultures were collected.

Table 1 Relative frequency of the dominant taxonomic classifications (more than 1%) of feces-isolated bacteria of the rabbits

a) Phylum	
Phylum	Relative Abundance
Firmicutes	66.42%
Verrucomicrobia	14.05%
Proteobacteria	9.54%
Unclassified at phylum level	6.87%
Bacteroidetes	1.54%
b) Class	
class	Relative Abundance
Clostridia	55.25%
Verrucomicrobia	14.85%
Unclassified Firmicute	7.90%
Unclassified at phylum	7.77%
Gammaproteobacteria	4.22%
Bacilli	2.68%

Betaproteobacteria	1.13%
Bacteroidia	1.03%
c) Order	
Order	Relative Abundance
Clostridiales	54.79%
Verrucomicrobiales	14.85%
Unclassified Firmicute	7.90%
Unclassified at phylum	7.77%
Pseudomonadales	1.89%
Bacillales	1.49%
Lactobacillales	1.19%
Xanthomonadales	1.17%
Bacteroidales	1.03%
d) Family	
Family	Relative Abundance
Unclassified Halanaerobiales (Firmicutes)	%20.77
Ruminococcaceae	20.03%
Verrucomicrobiaceae	13.92%
Lachnospiraceae	12.83%
Unclassified Firmicutes	7.29%
Unclassified Phylum	6.87%
Xanthomonadaceae	1.84%
Pseudomonadaceae	1.52%
Alcaligenaceae	1.08%
e) Genus	
Genus	Relative Abundance
Unclassified Clostridiales (Firmicutes)	20.77%
Unclassified Ruminococcaceae (Firmicutes)	10.86%
Persicirhabdus	9.32%
Unclassified Lachnospiracea (Firmicutes)	8.26%
Unclassified Firmicutes	7.29%
Unclassified Phylum	6.87%
Ruminococcus	4.93%
Unclassified Verrucomicrobiaceae (Verrucomicrobia)	2.66%
Akkermansia	1.94%
Ignatzschineria	1.46%
Clostridium_XlVa	1.23%

Table 2 Blood Culture Microorganisms

No	Microorganism
1	*S. aureus*
2	*S. faecium*
3	*S. pyogenes, C. albicans*

4	*K. pneumoniae, E. coli*
5	*S. typhi, A. hydrophila*
6	*P. aeruginosa*
7	*P. multocida*
8	*A. calcoaceticus*

Survival study

The rabbits in the group of 5.0 mL/kg survived for 16 days after sepsis induction, and the overall mortality rate following 14 days (OMR-14) of the induction of sepsis was 53% (16 out of 30 rabbits). In the group of 10 mL/kg with 30 rabbits, eight (26.7%) survived up to 24 hours after sepsis induction and the other 22 rabbits died following 36 to 72 hours of the induction of sepsis. Of 20 rabbits in the group of 15 mL/kg, seven (35%) survived up to 24 hours and four died following 24 to 48 hours of the induction of sepsis. The OMR-14 of this group was 55% (11 out of 20 rabbits). In the group of 20 mL/kg, none of the rabbits survived after 36 hours and the median survival time were 17 hours. According to the results of a log-rank test, the differences between the four groups in terms of the mortality rates were significant ($P < 0.001$). In addition, mortality was observed to increase in a dose-dependent manner. During the autopsy, multiple microabscesses and adhesion were noticed throughout the abdominal cavity (figure 1).

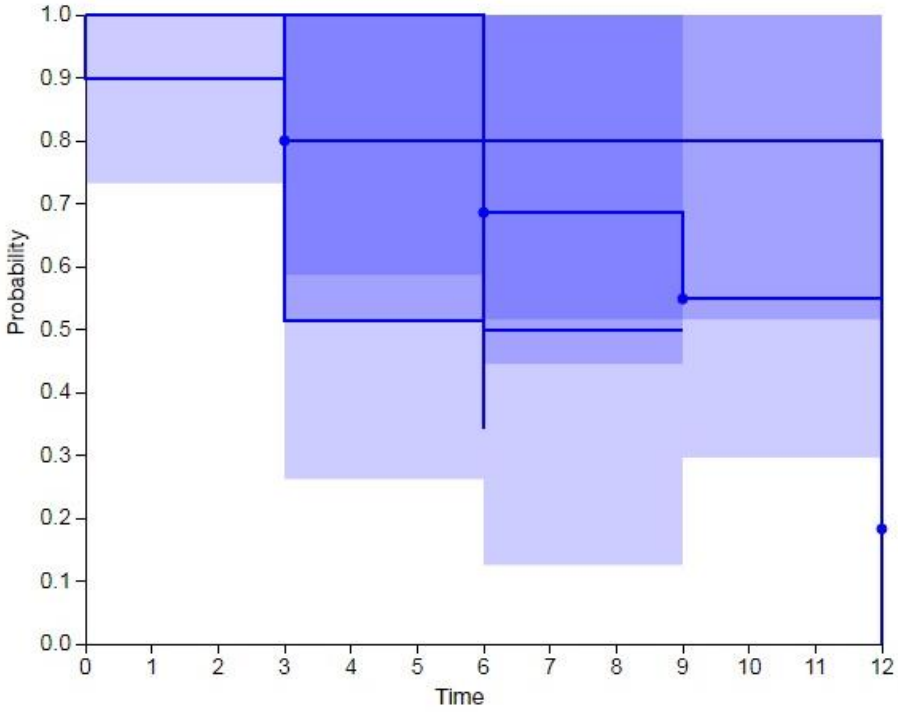
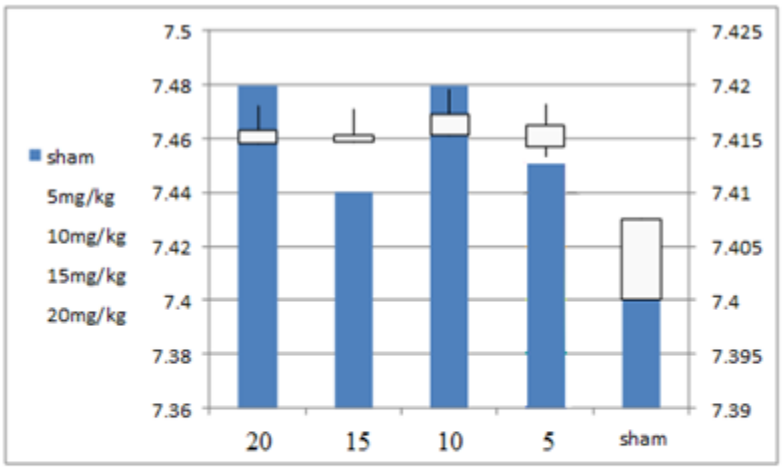


Figure 1 Kaplan–Meier survival curve. $P < 0.001$ by log-rank test for trend; $n = 10$ for 5.0 and 20 mL/kg groups; $n = 30$ for the 10 mL/kg group; and $n = 20$ for the 15 mL/kg group, respectively.

Analysis of arterial blood gas

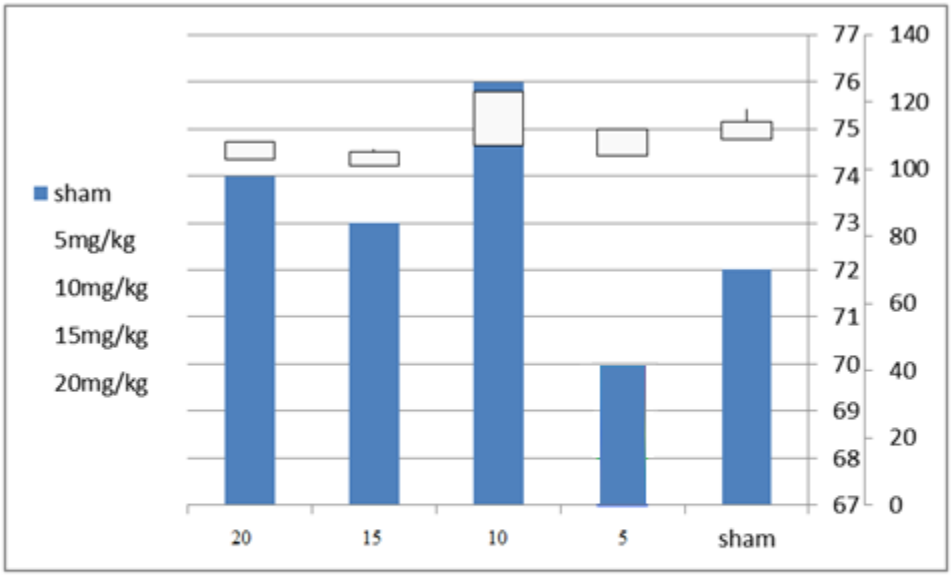
All sepsis groups showed significantly higher levels of Arterial pH and PaO2 than the sham group (figure 2A & B). On the other hand, lower levels of PaCO2 were observed in the sepsis groups than in the sham group. There was an inverse correlation between the dose of cecal slurry and the level of PaCO2, so that lower dose groups (5.0 and 7.5 mL/kg, figure 2C) showed higher levels of PaCO2 than the higher cecal slurry dose groups (10.0 and 15.0 mL/kg). Arterial blood HCO_3^- concentration decreased as the dose of cecal slurry increased, and these concentrations were both significantly lower in the sepsis groups than in the sham group. The concentration of arterial blood HCO3- had an inverse relationship with the dose of cecal slurry and was significantly lower in the sepsis groups compared to the sham group (figure 2D & E). In contrast, an increase in lactate concentration was observed with an increasing dose of cecum slurry (Figure 2F).



P-value * * * * -

A: PH

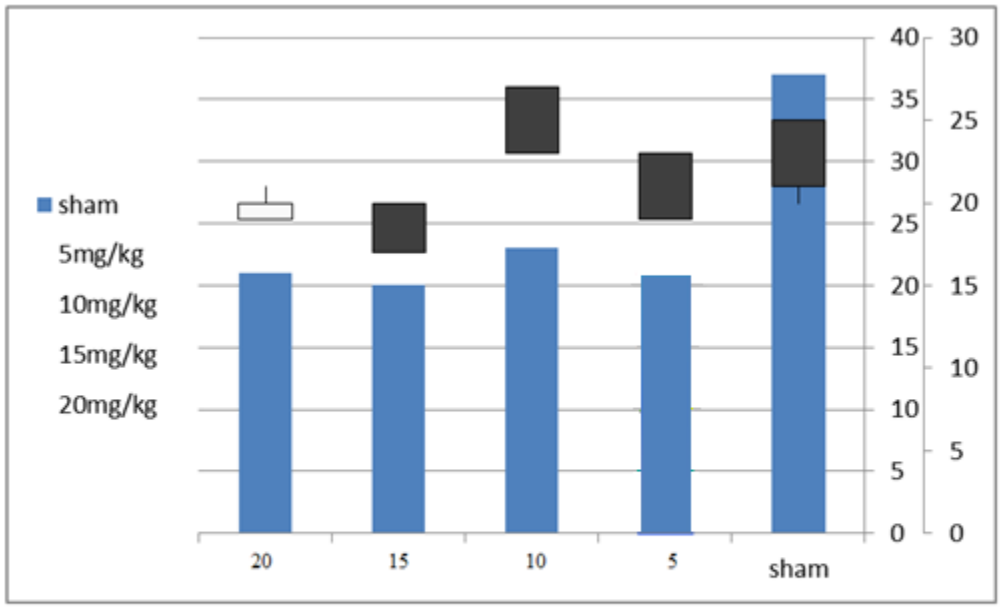
Fecal slurry (mg/kg)



P-value * * * * -

B: PaO_2

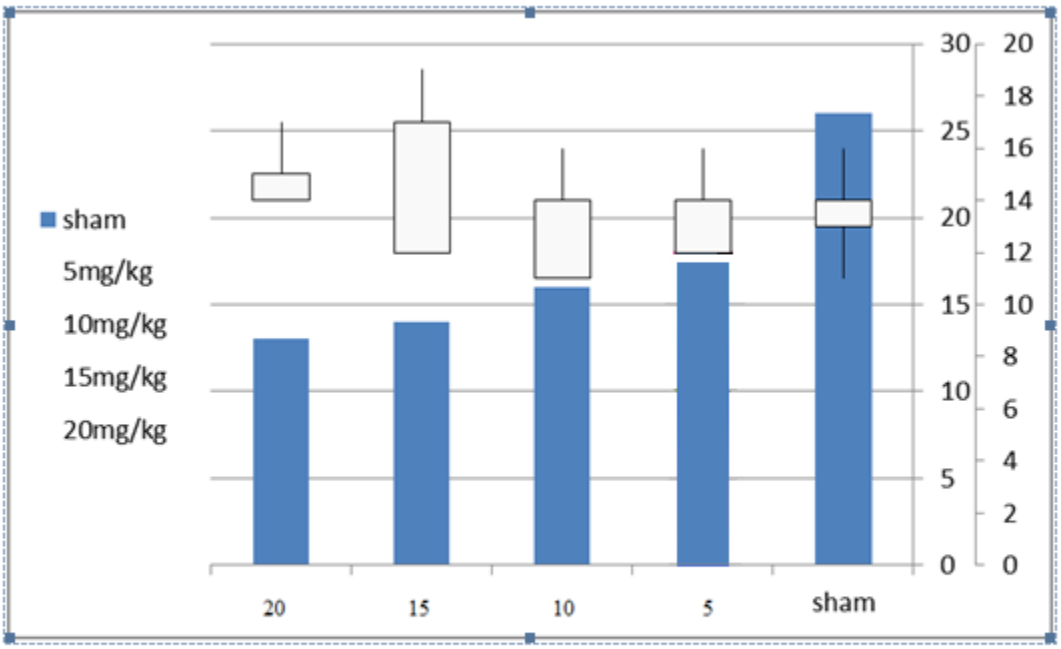
Fecal slurry (mg/kg)



P-value	***	***	*	*	-

C: $PaCO_2$

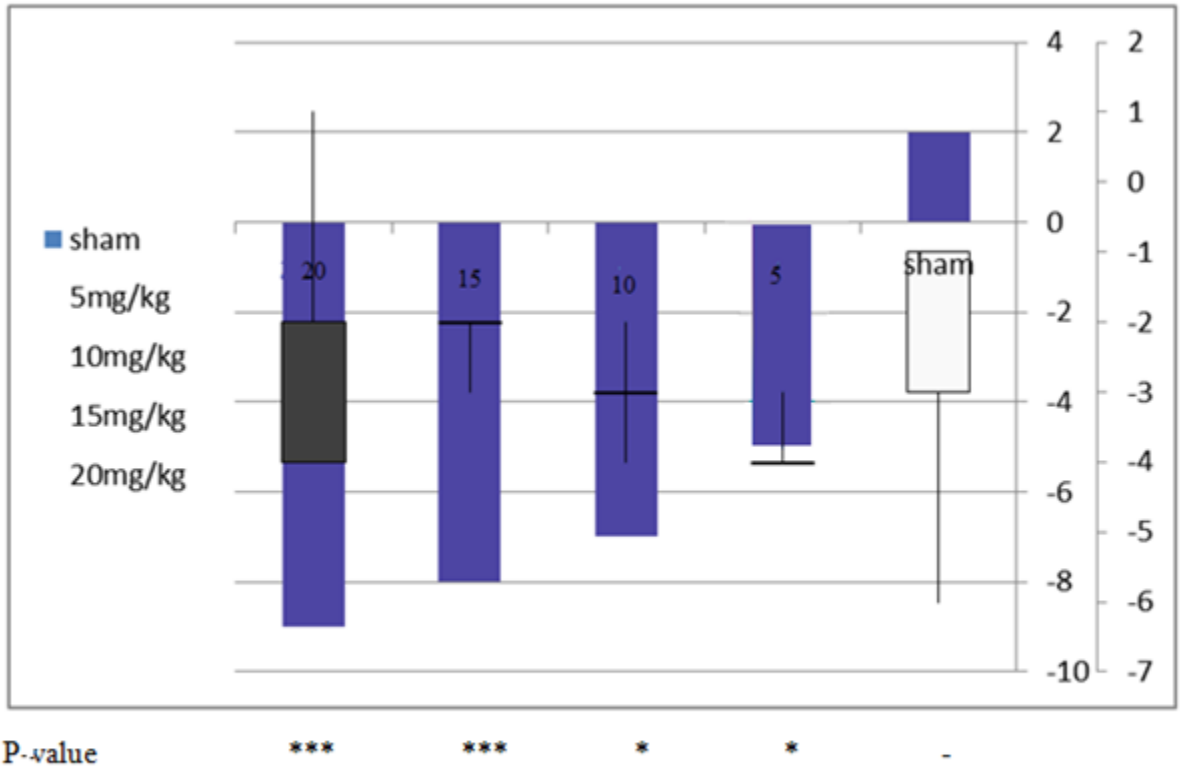
Fecal slurry (mg/kg)



P-value	***	***	*	*	-

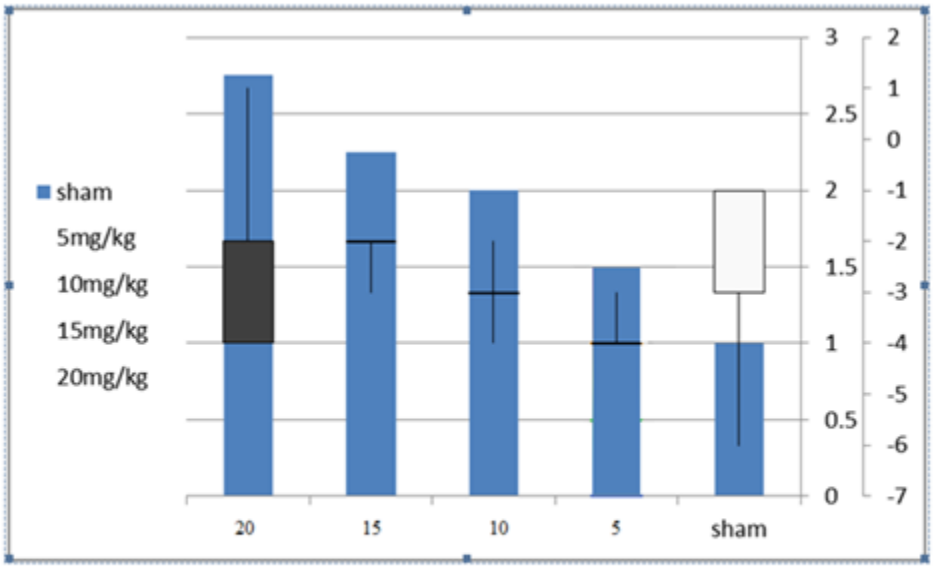
D: HCO_3^-

Fecal slurry (mg/kg)



E: Base Excess

Fecal slurry (mg/kg)



F: lactate

Fecal slurry (mg/kg)

Figure 2 Analysis of the arterial blood gas. (A) pH, (B) PaO2 concentration, (C) PaCO2 concentration, (D) HCO3− concentration, (E) base excess concentration, and (F) lactate concentration.

∗significant difference with the sham group ($P < 0.05$); ∗∗significant difference with the 5.0 mL/kg group ($P < 0.05$); #P significant difference with the 10 mL/kg group ($P < 0.05$)

n = 5 in the sham group and n = 10 in all sepsis groups.

Although the total number of lymphocytes, neutrophils, and white blood cells (WBC) in the groups of 15 and 20 ml/kg was less than the groups of 5 and 10 ml/kg, no significant differences were observed between the groups (P> 0.05) (table 3 & figure 3). Higher concentrations of hematocrit and hemoglobin were found in the sepsis groups compared to the sham group. The number of platelets was also higher in the 20 mL/kg group compared to the sham group (Figure 4 A- F).

Table 3 Rabbit Hematology and Blood Chemistry Reference Ranges

Hematology Ranges	
PCV	35 - 50 %
WBC	4 - 10 x10^3/μL
Heterophils	30 - 70 %
Lymphocytes	30 - 70 %
Monocytes	0 - 3 %
Basophils	0 - 1 %
Eosinophils	0 - 1 %
Platelets	250 - 650 x10^3/μL

Blood Chemistry Ranges	
Total protein	5.4 - 7.3 g/dL
Albumin	2.4 - 4.5 g/dL
BUN	10 - 33 mg/dL
Calcium	8.0 - 15.5 mg/dL
Cholesterol	10 - 80 mg/dL
Chloride	90 - 110 mmol/L
Creatinine	0.5 - 2.2 mg/dL
Globulin	2.9 - 4.9 g/dL
Glucose	80 - 150 mg/dL
Potassium	4.3 - 5.8 mmol/L
Sodium	140 - 160 mmol/L
Phosphorus	4.4 - 7.2 mg/dl
ALP	4 - 20 U/L
AST	10 - 120 U/L
ALT	10 - 45 U/L
Total bilirubin	0 - 1.0 mg/dL

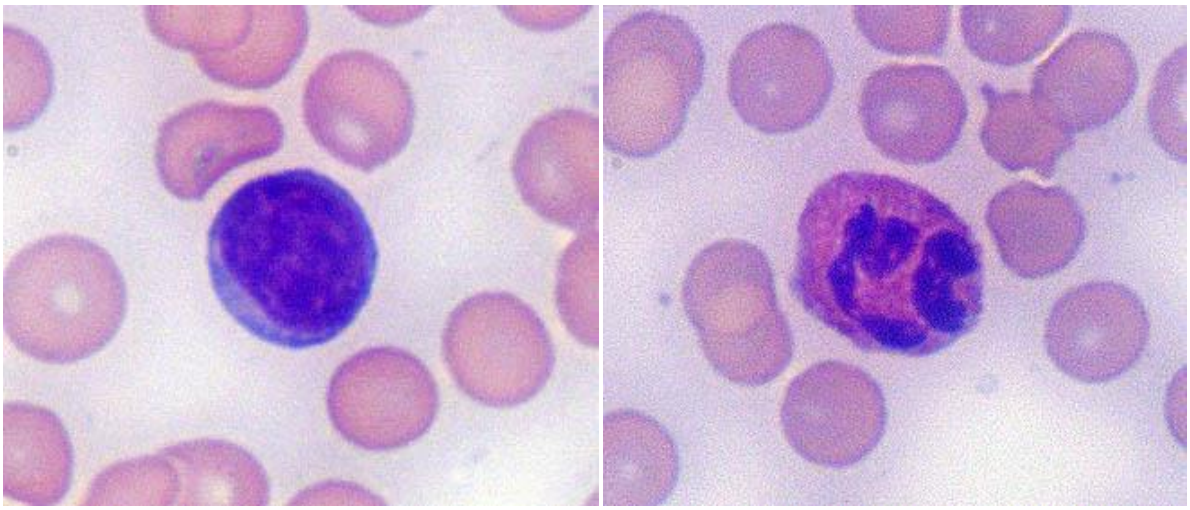
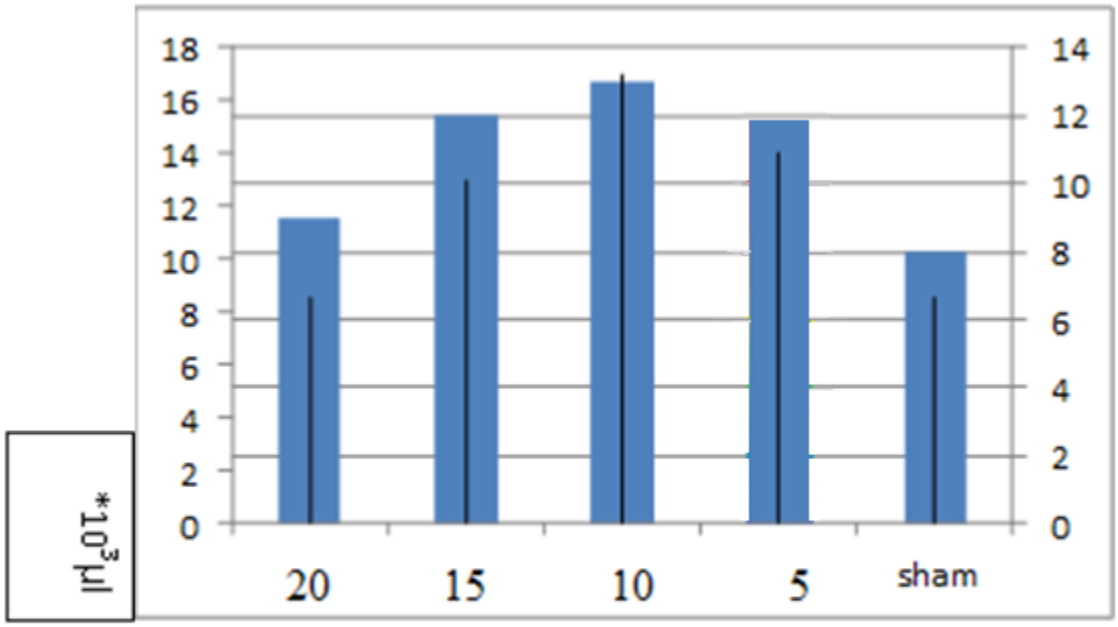
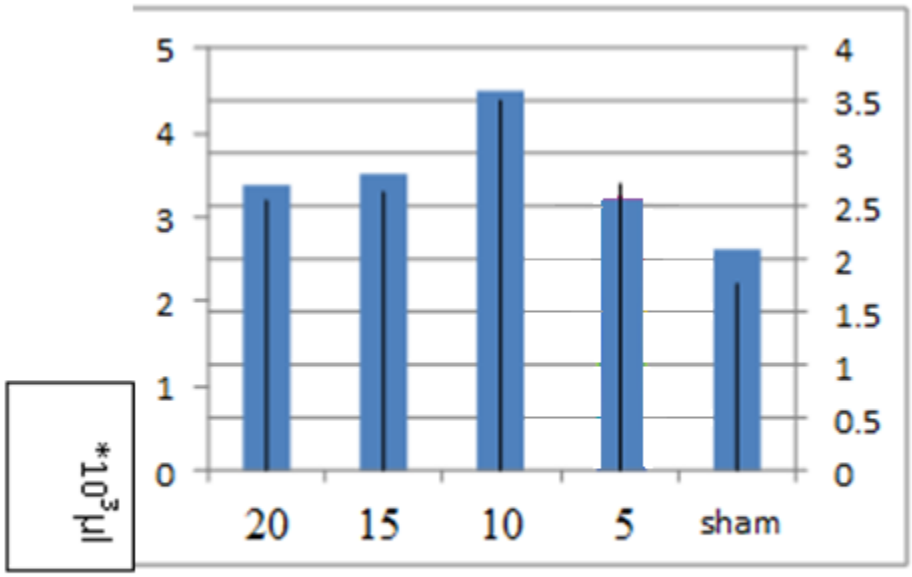


Figure 3 A normal, small, well-differentiated lymphocyte is on the left, Normal rabbit heterophils have a lobulated nucleus and small, diffuse, red, cytoplasmic granules (right).



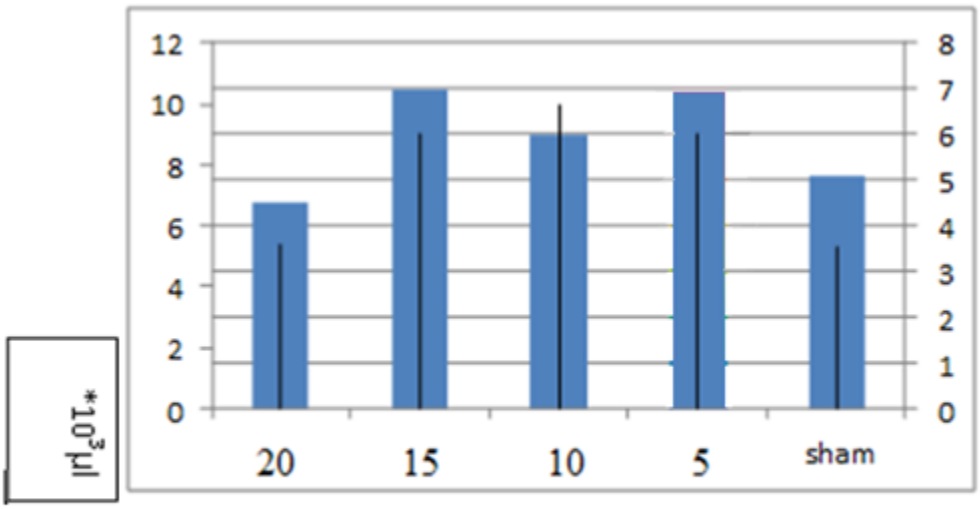
A: WBC

Fecal slurry (mg/kg)



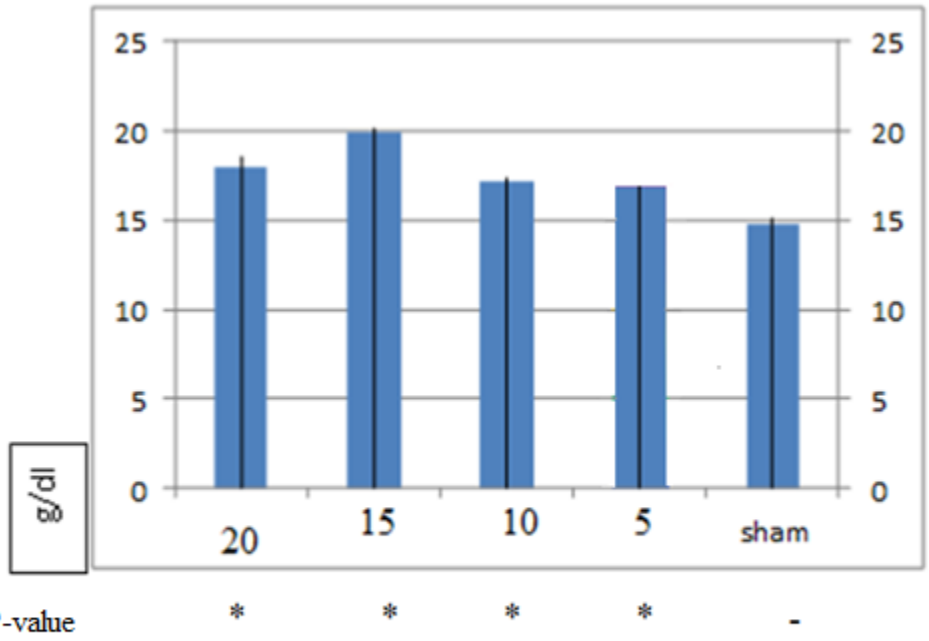
B: Neutrophil

Fecal slurry (mg/kg)



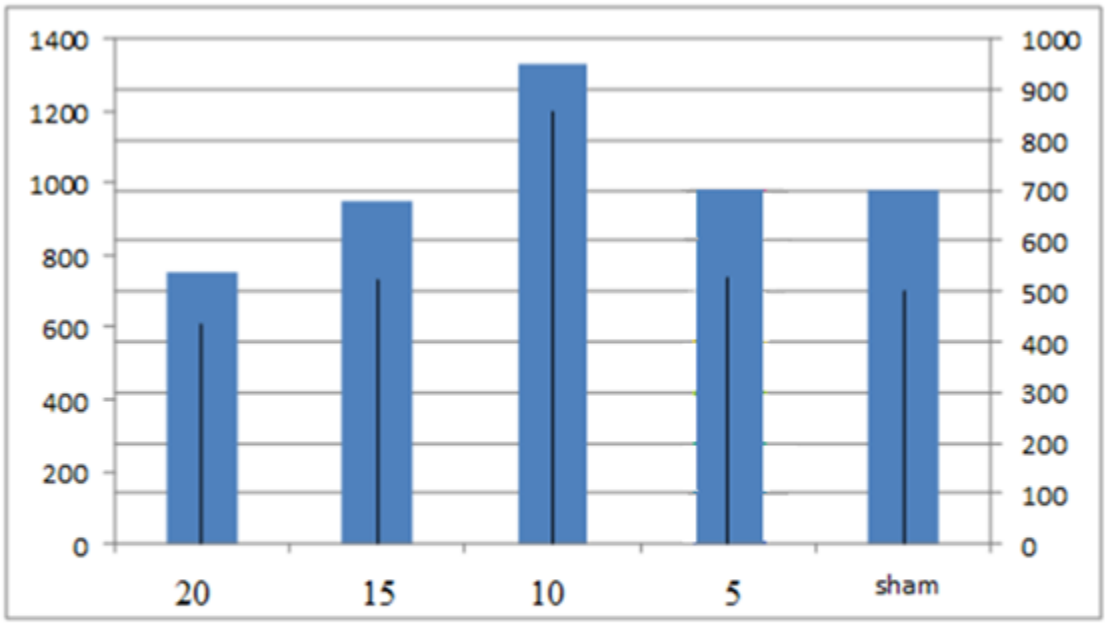
C: Lymphocyte

Fecal slurry (mg/kg)



D: Hemoglobin

Fecal slurry (mg/kg)



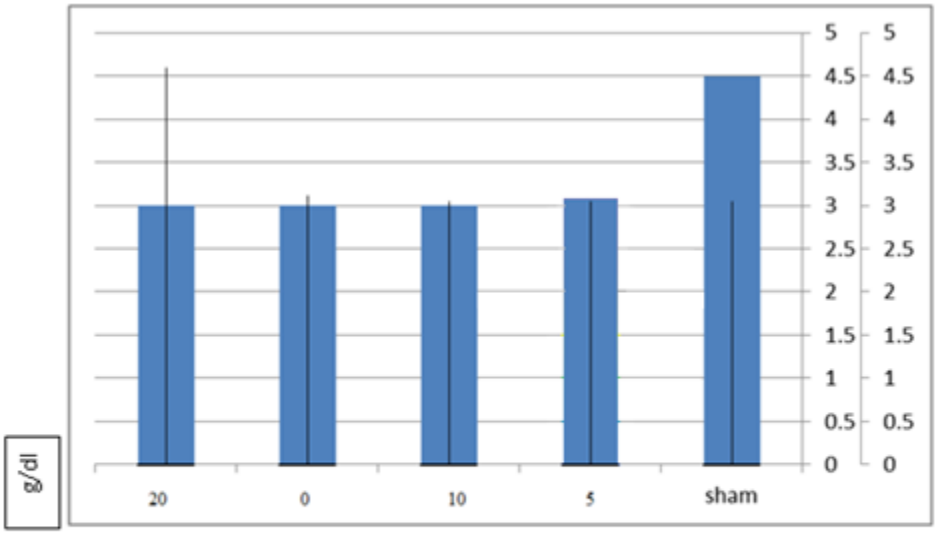
F: Platelet

Fecal slurry (mg/kg)

Figure 4 Complete blood count (A) the number of WBCs, (B) the number of neutrophils, (C) the number of lymphocytes, (D) the concentration of hemoglobin, (E) the concentration of hematocrit, and (F) the number of platelets. ⁎a significant difference with the sham group ($P < 0.05$); n = 5 in the sham group and n = 10 in all sepsis groups.

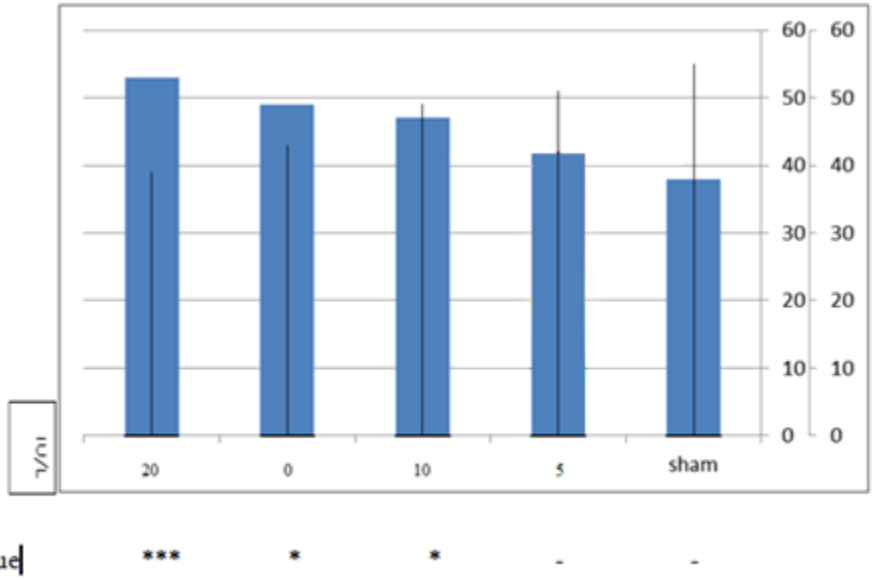
Plasma chemistry analysis

All sepsis groups had higher concentrations of plasma albumin compared to the sham group (Fig. 5A). There was a direct relationship between cecal slurry dose and the BUN and ALT concentrations, and significantly higher concentrations were found in the sepsis groups than in the sham group (Fig. 5B&C). Although higher concentrations of creatinine were found in the groups of 15 and 20.0 mL/kg than in the sham group, the differences were insignificant (Fig. 5D).



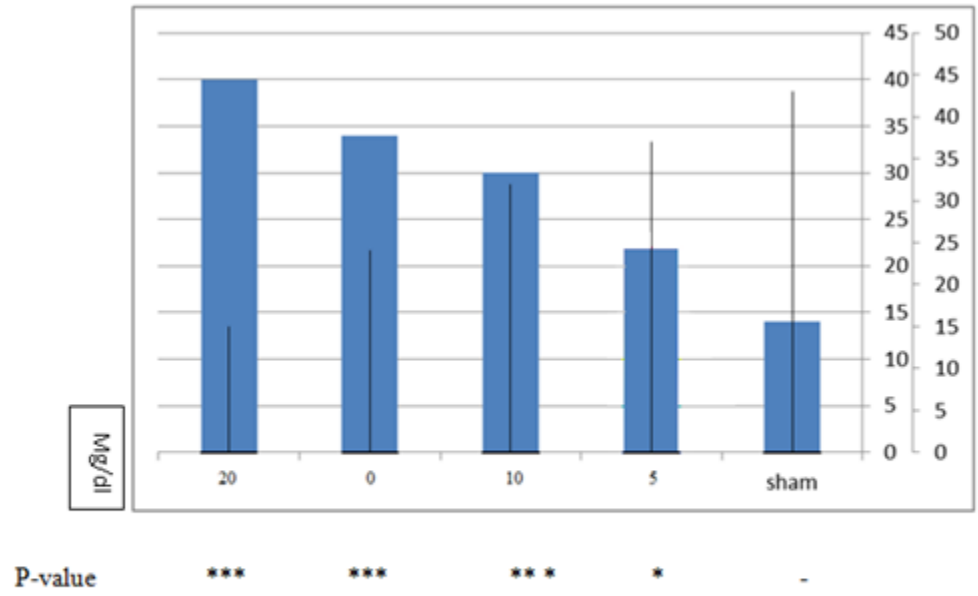
A: Albumin

Fecal slurry (mg/kg)



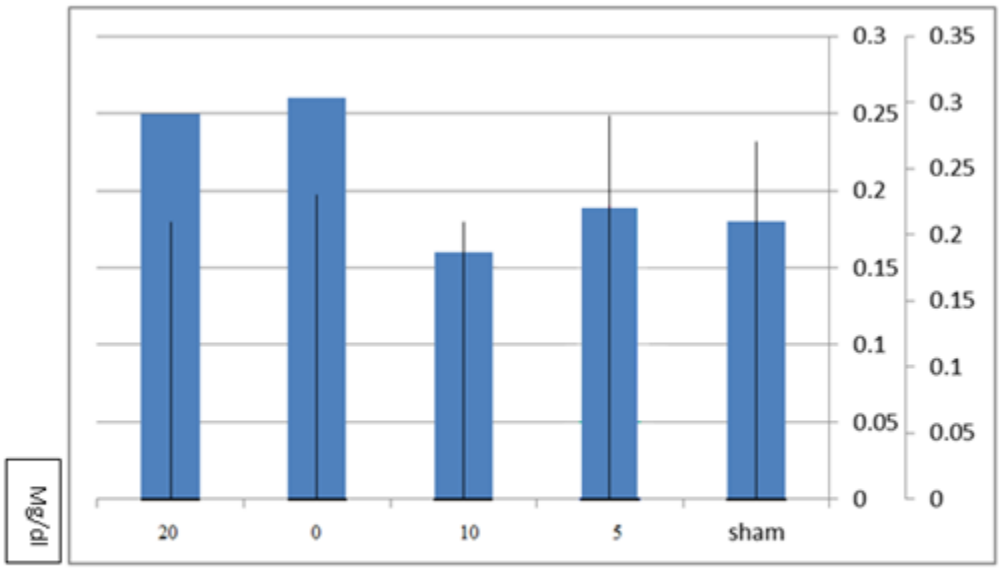
B: ALT

Fecal slurry (mg/kg)



C: BUN

Fecal slurry (mg/kg)



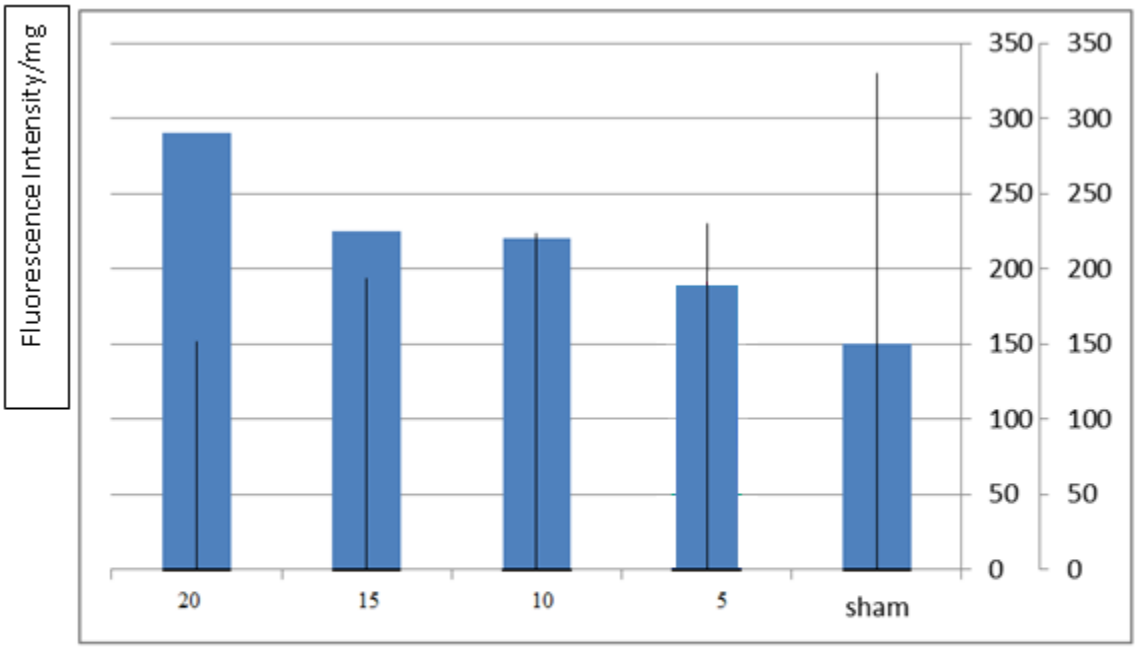
D: Creatinine

Fecal slurry (mg/kg)

Figure 5 Plasma chemistry analysis (A) Albumin concentration, (B) ALT concentration, (C) BUN concentration, and (D) creatinine concentration. ⁎significant difference with the sham group ($P < 0.05$); ⁎⁎significant difference with the 5.0 mL/kg group ($P < 0.05$); #P significant difference with the 15 mL/kg group ($P < 0.05$); n = 5 in the sham group and n = 10 in all sepsis groups. ALT=alanine aminotransferase; BUN=blood urea nitrogen

DCFH-DA in kidney, liver, and lung

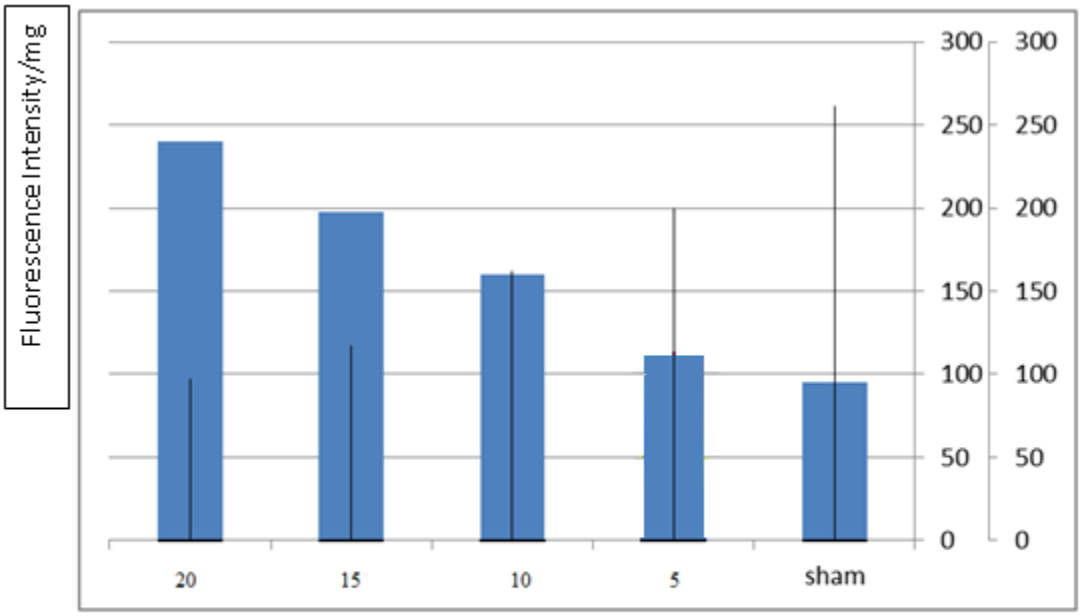
The concentration of DCFH-DA in the sepsis groups was significantly higher compared to the sham group (figure 6A-C). Moreover, higher doses of cecal slurry were associated with higher concentrations of DCFH-DA in kidneys, livers, and lungs (figure 6A-C).



P-value	***	***	*	*	-

A: DCFH-DA in Lung

Fecal slurry (mg/kg)



P-value	***	***	**	**	-

B: DCFH-DA in Liver

Fecal slurry (mg/kg)

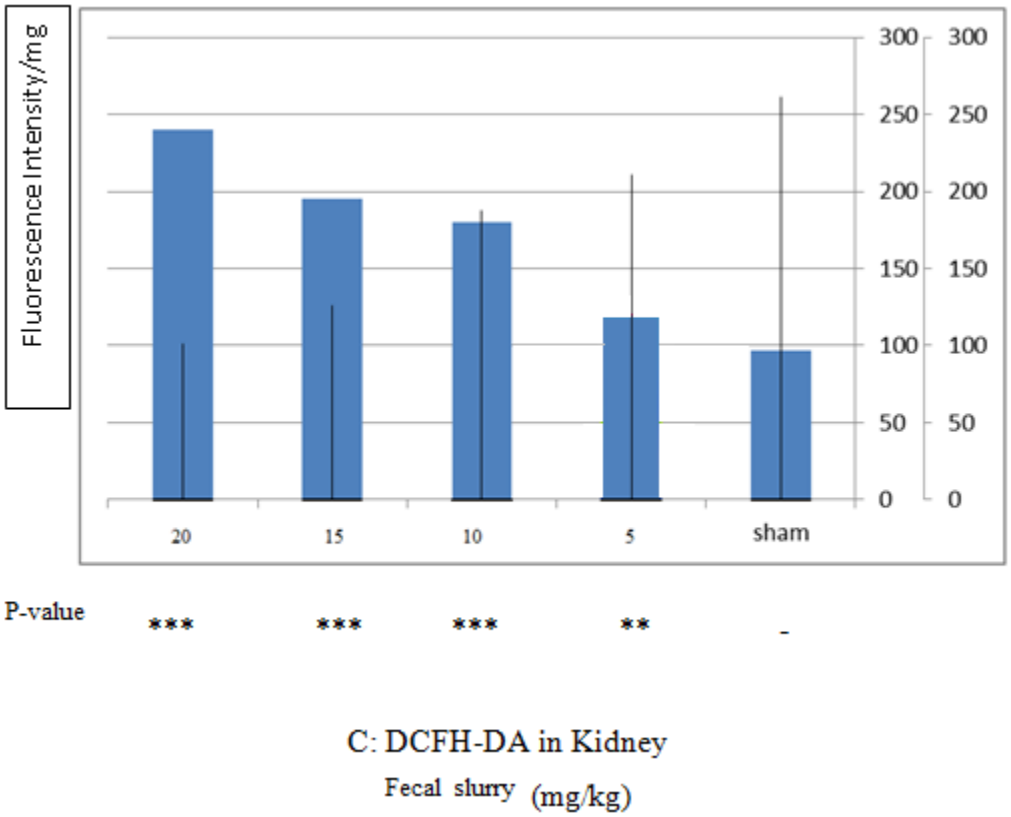


Figure 6 Dichloro-dihydro-fluorescein diacetate (DCFH-DA) in (A) lung, (B) liver, and (C) kidney tissues. ∗significant difference with the sham group ($P < 0.05$); ∗∗significant difference with the 5.0 mL/kg group ($P < 0.05$); #P significant difference with the 10 mL/kg group ($P < 0.05$); ##significant difference with the 15.0 mL/kg group ($P < 0.05$). n = 5 in the sham group and n = 10 in all sepsis groups.

4. DISCUSSION

In this study, the increase in infection was associated with a continuous and dose-dependent increase in mortality, the production of reactive oxygen species, metabolic acidosis, and pathological damage to organs (Campbell et al., 2012). Therefore, this study contributes to the existing knowledge about the infection process and its additive effect on each of these factors. Anesthesiologists face a variety of challenges when dealing with sepsis patients, both in the intensive care unit and in the operating room (Hufeldt et al., 2010; Kazarian et al., 1994). Yearly, 750,000 new cases of sepsis and 210,000 deaths from the disease are reported in the US. Almost 40% of patients admitted to intensive care units either are with sepsis at the time of admission or grow sepsis during hospitalization. Notable advances have been made in the comprehension of pathophysiology and the treatment of sepsis. Although the cases of sepsis-related deaths are still high, novel therapy algorithms have reduced overall mortality from this disorder (Buras et al., 2005; Hubbard et al., 2005; Starr et al., 2009; Starr et al., 2013).

One of the main obstacles to the development of a successful therapy for the treatment of sepsis is the lack of a truly relevant and predictive animal model with a clinical application for this disorder. In recent decades, scientists have developed and employed a variety of animal models of sepsis. Sepsis studies with animal models have sometimes been employed as precursors to introduce new therapeutic agents (Starr et al., 2011). However, animal models of sepsis have been applied more often as representatives for human bodies to provide better insights into the pathophysiology of the disorder. A large number of previous studies have documented various approaches in detail aimed at summarizing the main characteristics of septic shock and sepsis in patients. Accordingly, the present study no longer intends to address these issues (Maier et al., 2004; Itoh et al., 1987; Zanotti-Cavazzoni and Goldfarb, 2009; Wynn et al., 2007), rather it addresses the most common animal models used for sepsis in humans and focuses on the advantages and disadvantages of such models in developing pharmacological therapies for sepsis. Although is easy to apply and offers an acceptable response, the LPS model is unable to provide an accurate description of the complex nature

of sepsis and is therefore not regarded as a suitable model for sepsis in clinical applications (Saito et al., 2003; Wood et al., 1993; Lang et al., 1983).

Pathogenic bacteria have attracted much attention in modeling therapies for acute human infection using mouse models (Vincent et al., 2013). However, these models are not always associated with ideal results. For example, using Gram-negative bacterium *Pseudomonas aeruginosa* for acute infections in various animal models leads to a large inconsistency and higher infecting doses are rapidly lethal, while it resolves rapidly at lower infecting doses (Vincent et al., 2014; Seymour and Rosengart, 2015). One animal model widely used for sepsis is the CLP model, which uses perforated viscus to mimic polymicrobial sepsis. For the contents of the cecum to enter the peritoneal cavity in the CLP model, the cecum is punctured and ligated with a cannula. However, this is not a standardized procedure, since there is wide inter-operator variability in terms of the size of cannula and cecum, the number of punctures, the nature and number of cecal contents, and the time it takes for the cecal contents to diffuse into the peritoneal cavity (Starr and Saito, 2014; Nicholson et al., 2000). Such non-standardized factors can bring bias into the results obtained from this model. In addition, the CL model causes encapsulation or abscess formation in the animals instead of peritonitis, which raises concerns about the possibility of less severe sepsis in surviving animals (Noritomi et al., 2009; Miller and Nadon, 2000). Due to this, gaining reliable results based on sepsis severity is not easy.

As mentioned previously, CLP is a widely used animal model in research for inducing sepsis in laboratory species such as rabbits, mice, etc. (Dejager et al., 2011; Buras et al., 2005). This method is done by inducing polymicrobial peritonitis to anesthetized animals through cecal surgical ligation and subsequent needle puncture to allow cecal content secretions into the abdominal cavity. Since the CLP model is relatively easy to perform and produces close results with those obtained in the clinical course of intra-abdominal sepsis, many researchers prefer to use this model for studying sepsis in laboratory animals (Starr and Saito, 2014; Wichterman et al., 1980). Sepsis severity induced by the CLP model is a function of the cecal bacterial flora and the volume, duration, and secretion rate of cecal contents into the abdomen. Thus, CLP may not be the model of choice for experiments involving animals with various cecal shapes, sizes, or bacterial flora. This may be a limiting factor in the use of the CLP model in studies evaluating sepsis severity in animals of different sizes (e.g., mutant dwarf vs. neonatal mice) under different gastrointestinal conditions (e.g., old or young animals, or those with gastrointestinal pathology), diet regimens (e.g., diet restrictions, high-fat diet, or full-liquid diet), or different sensitivity to surgery (e.g., aged or defective wound-healing animals). In this study, the cecal slurry model was used to compare dose-dependent mortality rate and dose-dependent organ injury at a specific time point in rabbits (Turnbull et al., 2003, Starr et al., 2010).

According to the microbiological analysis, Firmicutes were the predominant phylum of bacteria within the completely gastrointestinal tract. In second place were Proteobacteria in the foregut and Bacteroidetes in the hindgut. A comparison of the live and total bacteria profiles showed a shift in the ranking of predominant phyla in the foregut. However, these changes were less obvious in the hindgut and slight variations were observed in the relative frequency of Bacteroidetes and Firmicutes. This finding is an indication of the more substantial interference of dead bacteria with the microbiota in the foregut. All 8 blood cultures obtained from the rabbits contained bacteremia. The isolation of *E. coli, S. faecium*, and *C. Albicans* were performed for each animal. The results indicated the necessity of prescribing another antimicrobial such as ampicillin or penicillin in combination with ceftriaxone affects both gram-negative and gram-positive microorganisms in the cecal slurry model. The survival rate was a function of the prescribed dose of cecum slurry.

The results of the survival study suggested that subsequent experiments on sepsis could be done by choosing a valid model with a specific severity. During the autopsies, adhesion and multiple microabscesses were noticed throughout the abdominal cavities, indicating the induction of diffuse peritonitis in this model instead of the formation of a localized abscess. Metabolic acidosis is a common phenomenon in sepsis and is associated with mortality. The results showed an increase in lactate concentration and a decrease in HCO3− and base excess with an increasing dose of cecum slurry. Furthermore, PaCO2 concentration decreased and pH increased in higher dose groups Overall, septic rabbits showed mixed respiratory alkalosis and metabolic acidosis in a dose-dependent manner. No such dose-dependent responses were observed for the WBC counts, and the group of 10 mL/kg showed the highest counts among the other groups. According to the results, a severe model of sepsis results in immune suppression, and the risk of leukopenia are possible in higher doses. Sepsis causes inflammatory responses that trigger subsequent damage to the organs.

The results also showed that higher doses of cecal slurry were associated with increased concentrations of ALT and BUN, indicating a dose-dependent manner of the liver and kidney injuries. Reactive oxygen species had a mediatory role in organ damage during sepsis. An increase in DCFH-DA concentration, which is a generator of reactive oxygen species, was observed in kidney, liver, and lung tissues.

5. CONCLUSION

Based on the DCFH-DA concentrations and blood chemistry analysis, the cecal slurry model used in this study produced dose-dependent, multiple organ injuries and was associated with reactive oxygen generation.

Abbreviation

(CLP) Cecal Ligation and Puncture
(LPS) lipopolysaccharide
(CFU) Colony-Forming Units
(ABG) Arterial Blood Gas
(BUN) Blood Urea Nitrogen
(ALT) Alanine Aminotransferase
(DCFH-DA) Dichloro-Dihydro-Fluorescein Diacetate

Consent for publication

All authors declare that they have Consent for publication

Authors' contributions

All authors contributed to the design of the study, as well as data collection and analysis, and the writing of the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are presented in the paper.

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